Nguyen et al – Supplemental material

Table S1. Accession numbers of *CITFA-7* orthologs

Species	Abbreviation	Accession number
Trypanosoma brucei	Tb	Tb927.7.2600
Trypanosoma congolense	Tco	TcIL3000.7.1910
Trypanosoma vivax	Tv	TvY486_0702470
Trypanosoma cruzi	Tc	Tc00.1047053506859.100
Leishmania major	Lm	LmjF.22.0680
Leishmania infantum	Li	LinJ.22.0550
Leishmania braziliensis	Lb	LbrM.22.0610

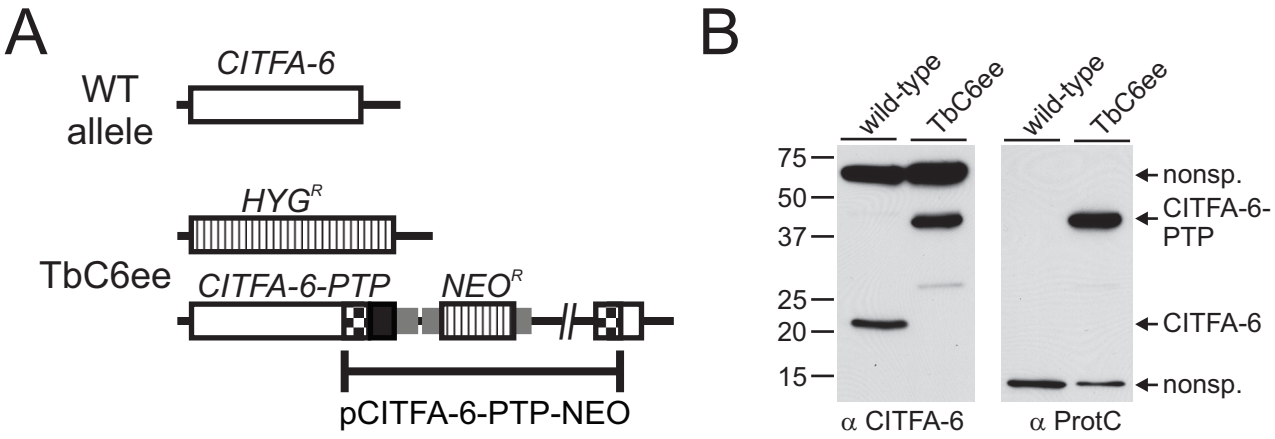

FIG. S1. Generation of cell line TbC6ee and an anti-CITFA-6 immune serum. (A) Schematic depiction of a CITFA-6 wild-type (WT) allele and of modified alleles in cell line TbC6ee which exclusively expresses CITFA-6-PTP and no untagged CITFA-6. The CITFA-6 coding region, *HYG*^{R} and *NEO*^{R} genes, and the PTP tag sequence are depicted by open boxes, striped boxes and a black box, respectively. Gene flanks for RNA processing signals are indicated by smaller grey boxes. (B) Immunoblot analysis of whole cell lysates of wild-type procyclic cells and of TbC6ee cells using a newly generated polyclonal anti-CITFA-6 immune serum (α CITFA-6) and a commercially available (Roche), monoclonal anti-ProtC antibody (α ProtC) that recognizes the PTP-tagged CITFA-6.

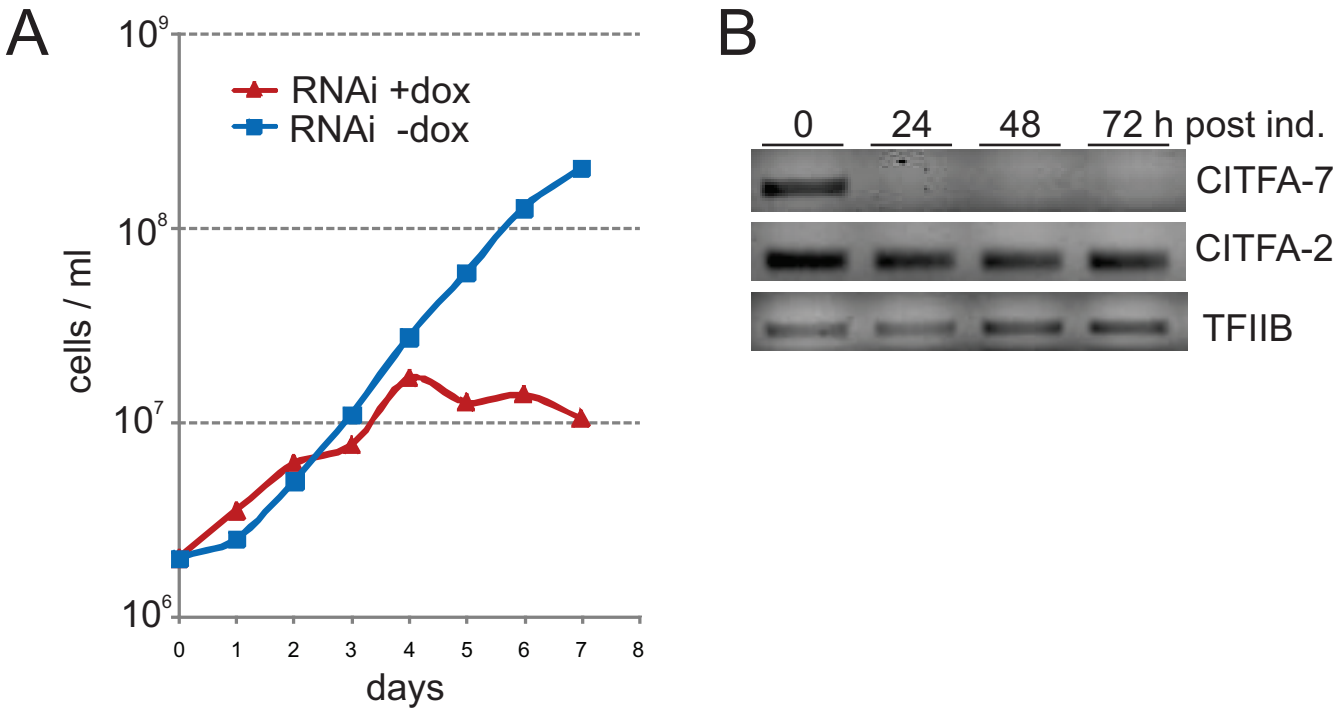

FIG. S2. *CITFA-7* silencing in a procyclic cell line. (A) Growth curve of a representative procyclic cell line in the absence or presence of doxycycline, the inducing compound for CITFA-7 dsRNA synthesis. (B) Semi-quantitative reverse transcription-PCR analysis of CITFA-7, CITFA-2 and, as a control, of TFIIB mRNA prepared from cells that were doxycycline-induced for the specified periods.

In procyclic trypanosomes CITFA-7 *silencing is lethal as in bloodstream forms (BFs) but, despite a very effective knockdown, cell death is delayed by at least 48 hours. The faster effect in BFs is most likely due to the inhibition of BF-specific VSG expression which was shown to very rapidly cause cell cycle arrest in this life cycle stage (Sheader et al., 2005).*

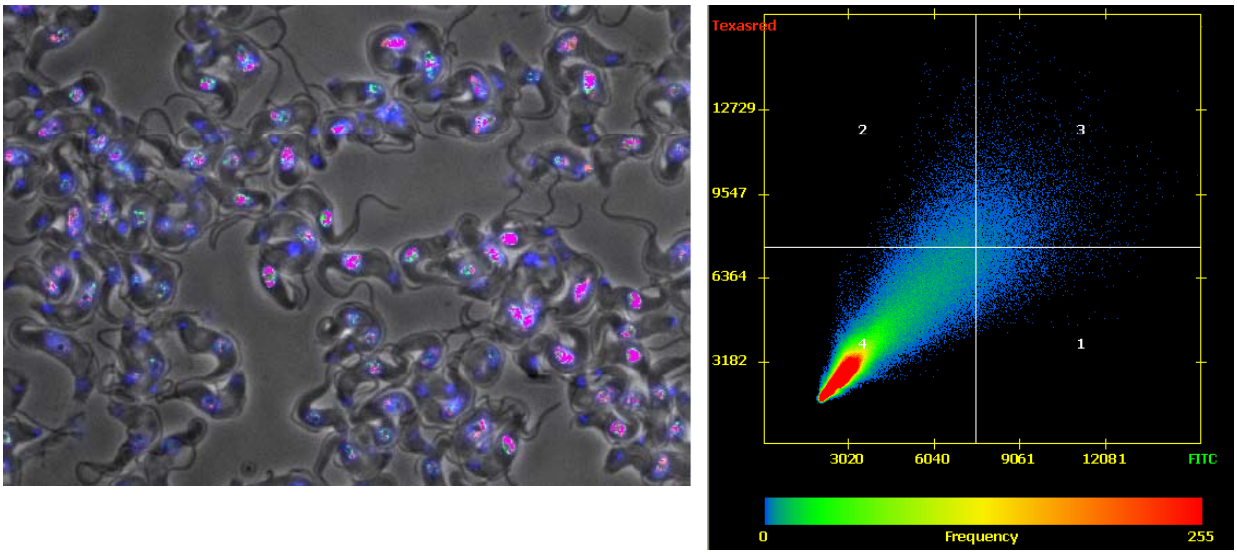

FIG. S3. Quantification of CITFA-7-HA/PTP-RPB6z (RNA pol I) co-localization. Left panel, representative picture of trypanosomes stained green (CITFA-7-HA), red (PTP-RPB6z), pink (co-localization) and blue (DNA, DAPI). Right panel , scatter blot showing non-co-localized CITFA-7 and RPB6z signals in quadrants 1 and 2, respectively, and high intensity co-localization signals in quadrant 3. Threshold was set to 7600, co-localization coefficient for Alexa 488 (CITFA-7-HA) was 0.48 and for Alexa 594 (PTP-RPB6z) 0.53.

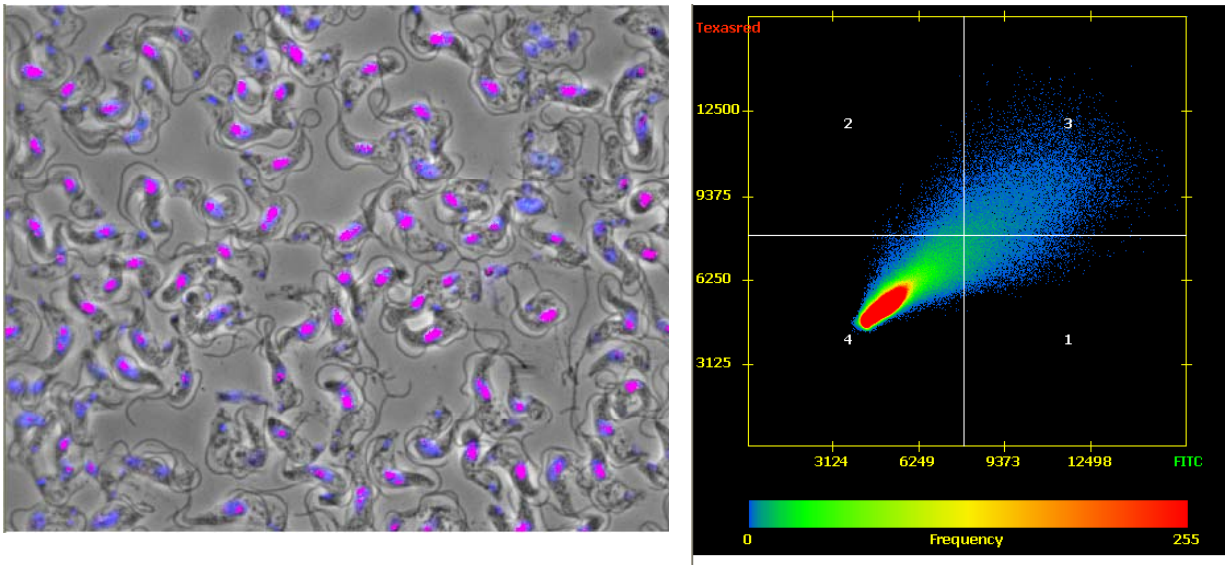

FIG. S4. Quantification of CITFA-7-HA/PTP-CITFA-2 co-localization. Left panel, representative picture of trypanosomes stained green (CITFA-7-HA), red (PTP-CITFA-2), pink (co-localization) and blue (DNA, DAPI). Threshold was set to 7900, co-localization coefficient for Alexa 488 (CITFA-7-HA) was 0.63 and for Alexa 594 (PTP-CITFA-2) 0.80.

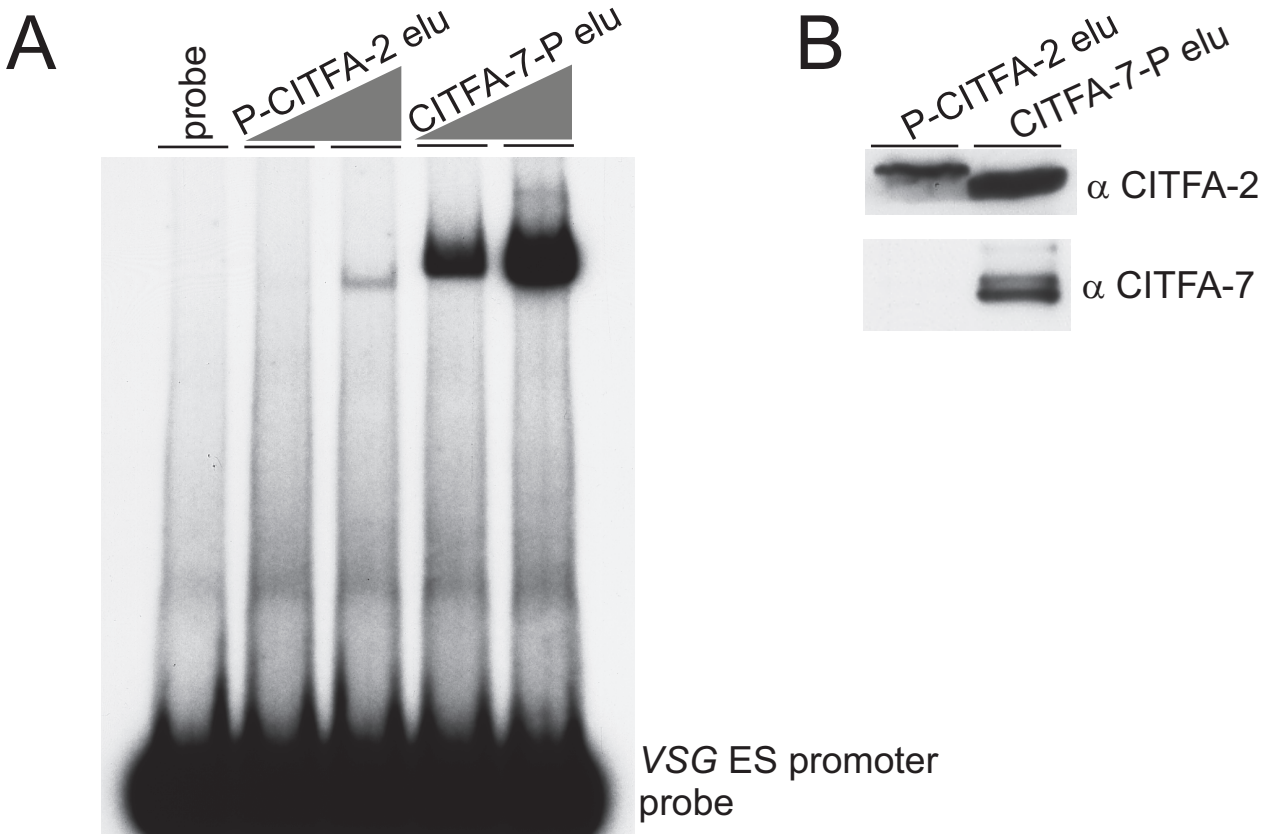

FIG. S5. *VSG* ES promoter binding efficiency of tandem affinity-purified CITFA-2 and CITFA-7 complexes. (A) A linear, radio-labeled *VSG* ES promoter fragment was shifted with final eluates of tandem affinity purifications of PTP-CITFA-2 (Brandenburg et al., 2007) and of CITFA-7-PTP (Figure 2D). For each eluate, two reactions with increasing protein amounts (1x and 4x) were carried out to demonstrate dose-dependence of DNA binding. (B) Immunoblot of the two eluates showing that they contain comparable amounts of CITFA-2 but not of CITFA-7.

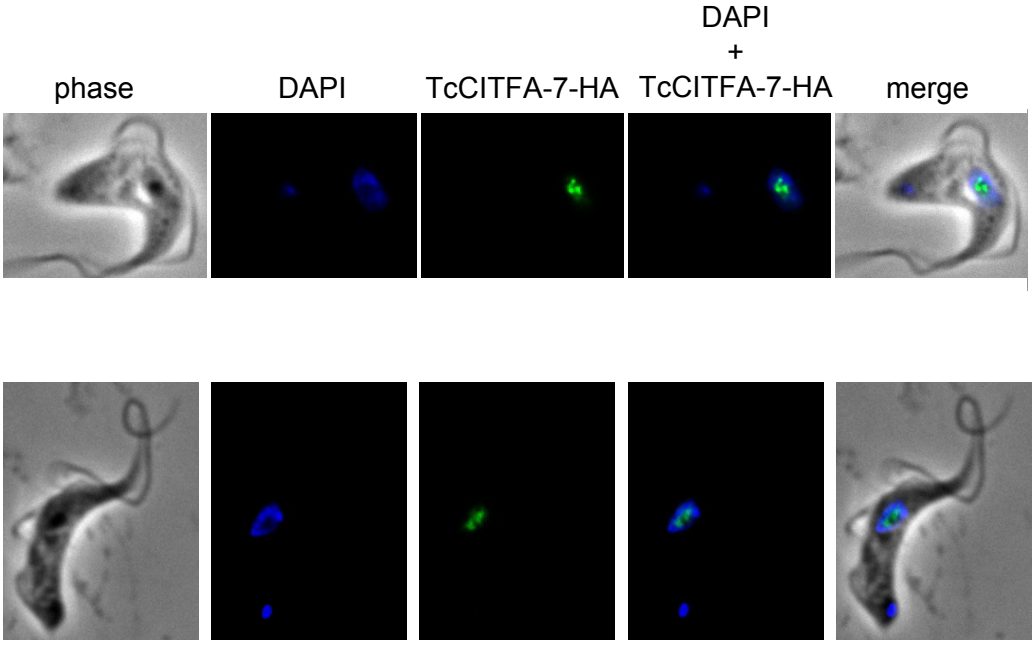

FIG. S6. Localization of *T. cruzi* CITFA-7-HA. Note that the nucleolus is the spherical structure of low intensity DAPI staining within the nucleus.

Supplemental References

Brandenburg, J., B. Schimanski, E. Nogoceke, T. N. Nguyen, J. C. Padovan, B. T. Chait, G. A. Cross, and A. Günzl. 2007. Multifunctional class I transcription in *Trypanosoma brucei* depends on a novel protein complex. EMBO J. **26**:4856-4866.

Sheader, K., S. Vaughan, J. Minchin, K. Hughes, K. Gull, and G. Rudenko. 2005. Variant surface glycoprotein RNA interference triggers a precytokinesis cell cycle arrest in African trypanosomes. Proc. Natl. Acad. Sci. U. S. A **102**:8716-8721.